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BROWN ADIPOSE TISSUE AND FOOD QUALITY IN RELATION TO POPULATION FLUCTUATIONS OF *Clethrionomys gapperi* AND *Peromyscus maniculatus*

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Brown adipose tissue and food quality in relation to population fluctuations of *Clethrionomys gapperi* and *Peromyscus maniculatus* submitted by Jean Meunier in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Inadequate amounts of the thermogenic brown adipose tissue (BAT) were postulated to be responsible for the increased adult and possibly neonate mortality that occur during declines in populations of small rodents living on the floor of the boreal forest. To test that hypothesis, trapping success and relative mass (as per cent of body mass) of interscapular deposit of BAT were studied quantitatively in an attempt to find if a correlation existed between the two. 178 *Peromyscus maniculatus* and 179 *Clethrionomys gapperi* were caught during June, July and August 1966 and May, June and July 1967. High positive correlations were found in both species during the 1966 population increase but correlations were low during the 1967 population decline except in female *C. gapperi* in which it was high and negative. No positive evidence of a relationship could be established between relative mass of interscapular BAT and survival of adults in summer. Relative volume of BAT was also studied in 12 *C. gapperi* embryos close to term and no significant difference was found between values in the two years. Finally, in an attempt to check if an inadequate diet during winter and an improved diet in spring (as presumed to occur in nature) could affect the mass of interscapular BAT, a mixture of laboratory chow and cellulose (1½:1 by mass) was fed during winter to 26 captive young female *P. maniculatus* followed in the spring, 11 days prior to autopsy, by either a shift to laboratory chow, a supplement of sprouted wheat or both. The winter diet failed to produce any apparent deficiency in mass of BAT.

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INTRODUCTION

Because the major physiological function of brown adipose tissue (BAT) is thermogenesis (Smith, 1964a) and because small rodents living on the floor of the northern boreal forest are exposed to microhabitat temperatures that seldom if ever rise above their lower critical temperature (Hayward, 1965), it seems reasonable to suggest that survival of small rodents may be related to their ability to maintain a supply of BAT. One purpose of this study was therefore to look for a correlation between amount of BAT and survival in wild *Clethrionomys gapperi* and *Peromyscus maniculatus* in a peak year and a decline year.

It is known that *C. gapperi* and *P. maniculatus* in the study area undergo rapid gain in body mass immediately following loss of the snow cover (Fuller, 1969) which may be related to a natural enrichment of the diet when new plant growth commences. A second purpose of this study was to duplicate experimentally this dietary shift to show whether an enriched spring diet is necessary for increased deposition of BAT.

Both study populations underwent declines in 1967 (Fuller, 1969). That year the appearance of the first litter was late and the number and size of litters born from overwintered females, as well as the body mass of the latter in early May, were low in the two populations as compared with the previous

years. These common differences suggest that extrinsic factors may have been involved. Weather, an extrinsic factor, appears to have contributed to population decline mainly by interfering with fall recruitment via a delay in onset of spring reproduction. However, there could also have been increased adult mortality. Krebs (1966) summarised Chitty's findings on mortality rates of *Microtus agrestis* and reported similar results with *M. californicus*. In both species of voles, mortality rates were low in winter; they increased markedly in spring about the time of onset of reproduction when they were specially high during decline years. Therefore the period extending from May to August was studied in the present work. In the study area it includes the spring critical period; in the months following the spring critical period microhabitat temperatures usually remain lower than the lower critical temperature of the animals (Fuller, Stebbins and Dyke, 1969).

Small laboratory rodents normally respond to cold acclimation by increasing their amount of BAT (Cameron and Smith, 1964; Pagé and Babineau, 1950; Smith, 1961; Smith, 1964b; Smith and Roberts, 1964). Yet, wild animals apparently have to undergo certain cold periods with a low amount of that tissue. Working with wild *M. pennsylvanicus* in the Edmonton region, Didow (1968) found that the amount of that tissue was markedly lower at the end than at the beginning of the 1967 winter. It seems that these voles traversed the spring critical period with a relatively low amount of BAT.

Elliott (1969) estimated at 12% per week the average mortality rate of *C. gapperi* from birth to trappable-size (5 weeks). The period he studied comprised breeding seasons in which population numbers were relatively similar. The extent of that mortality could be greater in years of population decline. It has been suggested that BAT is involved in resistance of newborn laboratory rats (*Rattus norvegicus*) (Smith, 1964a) and rabbits (Dawkins and Hull, 1964; Hull and Segall, 1964) to cold temperatures. In newborn lemmings (*Lemmus lemmus*), microtines of boreal origin, BAT is present at birth (Hissa, 1968) and the animals exhibit early thermoregulation whereas in hamsters (*Mesocricetus auratus*), cricetines of tropical origin, immature BAT cells appear on day 3-5 (Smalley and Smalley, 1967) and thermoregulatory response only on day 10 (Hissa, op. cit.). It seems reasonable to assume that without an adequate amount of that tissue, animals would be incapable of producing sufficient heat and would die. Neonates are not trappable and large embryos ($\geq$ 17 mm) from females close to parturition were utilised. Study of prenatal rather than postnatal animals avoided at the same time the bias of studying only survivors of an assumed postnatal loss.

Mammals have two types of fat that differ in their structure and function (Hull, 1966): white and brown adipose tissue. In mature white adipose tissue cells the nucleus and cytoplasm are confined to the periphery and a single lipid vacuole occupies the center of the cell. In normal BAT cells

the nucleus is central, the cytoplasm is granular and contains multiple fat droplets. BAT deposits have a dark color sometimes approaching that of the liver and a discrete lobular form. Generally, the anatomical distribution of BAT varies only slightly from species to species, the bulk of the tissue being located in the anterior part of the animal (Fig. 1). Didow (1968) recorded ten deposits in the meadow vole (M. pennsylvanicus), only one of which (renal) was located in the abdomen; the abdominal deposits also account for a small fraction of the total in the deer mouse (P. maniculatus) (Rauch, 1968). The group of all deposits (Rasmussen, 1923) or the interscapular deposit (Smith, 1964a) are sometimes referred to as the hibernating gland. However, in the majority of species investigated, those deposits do not show the histological structure of a gland and a variety of non hibernators possess it (Rasmussen, op. cit.).

The interscapular deposit of BAT has been utilised for diverse purposes including that of an index to the total amount in many studies (Aronson et al., 1954; Hissa, 1968; Lemonde and Timiras, 1951; Pagé and Babineau, 1950; Selye and Timiras, 1949; Smith, 1961). After Pagé and Babineau (op. cit.) had reported that the mass of the interscapular BAT increased in cold acclimated laboratory rats, Smith and Roberts (1964) showed that this increase affected all major sites. Similar results are reported in Smith (1964b). The latter study also showed that the interscapular deposit was the largest of all deposits and increased from .2 to .3% of body mass during cold acclimation

of rats. It can be seen from another study (Cameron and Smith, 1964) concerning the same species that the interscapular deposit constituted aproximately 40% of the mass of the deposits measured and that temporal fluctuations in both the mass of interscapular deposit and the sum of major deposits followed a similar pattern. It appears from these studies that changes in mass of the interscapular deposit are a reliable index to changes in mass of total deposits in adult laboratory rats. The same characteristic was assumed for the interscapular deposit of the study species. The fat concentration of that tissue was also presumed to be relatively constant as in wild *M. pennsylvanicus* (Didow, 1968).

STUDY AREA

The study area (Fig. 2) is situated in the District of Mackenzie, Northwest Territories. The forest is described in Rowe (1959) and consists of a mixture in varying proportions of white spruce (*Picea glauca*), jackpine (*Pinus banksiana*), black spruce (*Picea mariana*) and occasional trembling aspen (*Populus tremuloides*). Trapping was done in the forest bordering the Mackenzie highway from Mile Post 50 to Mile Post 85. A description of the weather in the area during the periods covered by the present work and an analysis of its implications in the life of the study animals were made by Fuller (1969).

MATERIALS AND METHODS

Snap-trapping and autopsies

Snap-trapping was carried out during June, July and August 1966 (4,439 trap-nights) and May, June and July 1967 (6,840 trap-nights). Trapping success, expressed as number of animals captured per 100 trap-nights was utilised as an index of population density. Set in the evening, traps were visited daily in the mornings, reset whenever necessary and picked up on the third day. No attempt was made to correlate type of vegetation with trapping success. Species other than C. gapperi and P. maniculatus were caught occasionally but are not included in the present work.

Autopsies were made at Heart Lake Biological Station, University of Alberta. The animals were weighed and their mass recorded; the mass of the pregnant females included the mass of embryos. Embryos were counted and the crown-rump length of one (in its membranes) in each set was measured. Ovaries as well as reproductive tracts and their content were preserved in Bouin's solution for later study. Unless damaged, organs and anatomical parts including skull, testes, stomach, spleen, kidneys and adrenals were preserved for concurrent studies. Interscapular BAT was identified by its position between the scapulae, its lobular form and its brown color; the pad was

removed and weighed immediately. Its relative mass expressed as per cent of body mass was used throughout the present study.

Selection, preparation and examination of *C. gapperi* embryos

Study of BAT in embryos was limited by the material available: in 1966 (peak), six and in 1967 (decline), three of the pregnant *C. gapperi* caught had embryos measuring 17 mm or longer. Two embryos were examined from each of three of the 1966 litters and the three 1967 litters. Litter sizes ranged from two to seven (Table 3).

After removal from Bouin's, the embryos were freed from their membranes and their length, mass and volume were measured. No morphological sex determination was attempted. To insure adequate infiltration, limbs were cut off and the embryos were cross-sectioned anteriorly at the level of the eyes and posteriorly at the level of the umbilical cord and the end parts were discarded (Fig. 4). The twelve embryos midsections were then submitted to identical histological treatments. Conventional paraffin method was used; blocks were sectioned serially on a rotary microtome (AO Spencer, Model 820) at a thickness of 10 μ, resulting in a relatively rugged ribbon. A sample consisting of every tenth section (Fig. 9) was affixed to embryological slides and stained with Mallory (Cason, 1950). The rest of the ribbon was stored for further reference. Two newborn hamsters

(*Mesocricetus auratus*) were processed in the same manner for gross anatomical comparisons (Fig. 10).

Tissue containing vacuolated cells and located at the sites where BAT is found in adults was assumed to be BAT. The same assumption was made of tissue situated elsewhere but having a similar histological appearance. That tissue stains magenta, has a discrete lobular form and is, in most cases, traversed by or closely associated with one or more blood vessels. Nervous tissue may have a similar colour but can easily be distinguished morphologically.

The areas of the sample sections showing BAT were measured with a calibrated ocular grid under a dissecting microscope. From the values obtained, the absolute volume of BAT in each deposit was calculated, knowing the thickness of the sample sections and their sampling pattern and assuming that each section was identical to the ones immediately adjacent to it in the embryo. An arbitrary relative value of BAT in relation to body mass was obtained by dividing volume of BAT in cubic centimeters by body mass in grams; if a specific gravity of 1 is assumed for embryos, that value expresses the relative volume of BAT as per cent of body volume.

Food experiment with *P. maniculatus*

Twenty-eight female *P. maniculatus* weighing an average of

13.76 ± 0.47 g were live-trapped between 5 and 12 August 1967 and brought back to the laboratory. Despite constant trapping efforts throughout August, C. gapperi could not be captured in sufficient numbers to build a colony because of their low population numbers.

Animals were marked by toe-clipping and introduced into cages in groups of two. Cages made of ¼ inch hardware cloth with a steel top, measured 10 × 20 × 25 cm. Each one was separated transversely by a ¼ inch thick wood partition into a nesting and a feeding compartment; the former was filled with terylene batting (Western Fibers Co., Vancouver) and the latter was provided with water and an aluminum feeder 3 × 3 × 20 cm. The partition did not extend the entire width of the cage so that mice could travel freely from one compartment to the other. Cages were numbered and kept on racks (Fig. 5). Temperature was maintained a few degrees above freezing by means of a thermostatically controlled electric heater and the animals were exposed to the natural photoperiod of the Edmonton region.

Pairs were randomly distributed into five unequal groups (Table 4); during winter, one animal died in group 1 and one in group 2. Pelletted Vitamite Cubes (North West Feeds Co., Edmonton) were supplied ad libitum to all groups until December. Then group 1 was transferred to a diet of ground Vitamite Cubes while groups 2, 3, 4 and 5 were fed ground Vitamite Cubes mixed with pure cellulose (Nutritional Biochemicals Corp., Cleveland) in the proportion of 3:1 by mass. In the latter groups, the

bulkiness of the diet was then gradually increased by successively bringing the ratio to 2:1 and 1½:1 (Fig. 16). On 10 May, a new diet was given to three groups: ground Vitamite Cubes replaced the bulky diet of groups 2 and 3 and sprouted wheat was added to the diet of groups 3 and 4. Eleven days later, the animals were sacrificed. Mass of body and interscapular BAT were recorded. Reproductive tracts and ovaries were examined. Fat deposits around the ovaries suggesting absence of any energy deficiency despite the dietary manipulations, made macroscopic count of corpora lutea difficult; therefore ovaries were fixed in Bouin's solution and a few were processed for histological examination. All animals were weighed at relatively regular intervals throughout the experiment.

The statistical methods used in the present study are from Simpson, Roe and Lewontin (1960). Material collected and information recorded will be deposited in the collection of the Department of Zoology, University of Alberta.

RESULTS

Clethrionomys gapperi

Wild population

If interscapular BAT is proportional to size of animals, correlation coefficients should be positive ($r_A > 0$) between

absolute mass of that tissue and body mass and close to zero ($r_R \approx 0$) when relative mass is considered. Until mid June, when populations consisted exclusively of overwintered adults (Fuller, 1969), the relationships described above were observed only in late May and early June with females and in early May with males (Fig. 6). In early May, the difference between values of coefficients in the two sexes was paralleled by a similar significant difference in body mass (Fuller, op. cit.).

After mid June the population consisted of animals of mixed ages and coefficients were predominantly positive with the absolute mass of interscapular BAT and negative with its relative mass. If body mass is used as an index of age, these coefficients suggest that absolute and relative mass of interscapular BAT were respectively lower and higher in juveniles than in adults. During the summer months relative mass of BAT is also higher in juvenile M. pennsylvanicus than in adults (Didow, 1968). The late July and late August drops in the values of the coefficients in males were probably not the result of the appearance in the population of young because they did not occur in female coefficients.

Population numbers in 1966, as revealed by snap-trapping, were slightly higher in males than in females but the pattern of fluctuation was roughly similar in both sexes (Fig. 7). These data suggest a decline in late August; however, live-trapping on permanent plots indicated that the population continued to rise

in that period (Fuller, 1969). A sharp decline with some recovery in midsummer was revealed by both trapping methods in 1967. In males, that decline occurred later and was not followed by recovery.

Grouping of the data to include the unsuccessful traplines for which there was no corresponding value of relative mass of interscapular BAT, made semimonthly periods the smallest units that could be used in Figure 7. In both years relative mass of interscapular BAT fluctuated, in general, less than trapping success. In 1966, it showed a clear trend to increase in both sexes. With females, the late August values were significantly higher than those for either late June or early July (Table 1). With males, early August values were significantly higher than late June and early July. Although late August values were higher still the sample was too small to yield a significant comparison. One possible explanation for those increases may be rejected. Didow (1968) found in _M. pennsylvanicus_ that the marked fall increase in relative mass of BAT corresponded with the first appreciable drop of temperature; such a drop at Hay River Airport took place only in September (Fig. 3). Consequently, the August 1966 increase was probably not due to temperature. However, it took place while there was a regular input of juveniles into the population, a situation that was likely to produce the rise observed since relative mass of interscapular BAT was higher in juveniles than in adults as indicated earlier (Fig. 6).

In 1967, a significant decrease in relative mass of interscapular BAT took place between late May and early June in females and between early and late May in males creating in that last period a significant difference between the values in the two sexes. Diverse stressors (Lemonde and Timiras, 1951; Selye and Timiras, 1949) including reproduction (Trusler et al., 1953) are beleived to affect the level of that tissue in small rodents. Spring changes related to body mass and to r_A correlation coefficient also took place earlier in males than in females. The time of occurrence of these changes and their precedence in males suggest a possible connection with the onset of reproduction. The subsequent higher levels of interscapular BAT in the survivors of the concomitant decline in population numbers could reflect the reaching of the "resistance phase" (Lemonde and Timiras, op. cit.), the higher level of the tissue in animals that survived, or both.

Besides the brief BAT decrease just mentioned, there were no other significant differences between semimonthly values over major parts of the trapping season in either sex or between values in the two sexes at the end of the trapping season. Therefore, despite the apparent different directions taken in the two sexes by the values of relative mass of interscapular BAT, the presence of any trend in that trapping season is rather doubtful. Finally the relative mass of interscapular BAT in both sexes was significantly lower in early May 1967 than in late August 1966. Whatever led the relative mass of interscapular BAT

to fluctuate, increases in semimonthly mean values were paralleled by population increases in 1966, whereas absence of trends corresponded with population decreases in 1967.

Because of the grouping method already mentioned, only five (1966) or six (1967) points could be utilised to compute correlation coefficients between relative mass of interscapular BAT and trapping success (Fig. 8). With females, that coefficient was negative and close to significance in 1967 but it was positive and low in all other cases. It has already been suggested that snap-trapping indices for late August did not agree with the population trend revealed by live-trapping. If these disputed values are left out, correlation coefficients become 0.88 and 0.76 for males and females respectively, values close to significance.

Embryos

The first and last sections of C. gapperi embryos showed no BAT (except for a negligible amount in one case). Distribution of deposits in the embryos under study was similar to that found in the neonatal laboratory mouse (Fig. 1) by Cameron and Smith (1964) and their terminology was adopted in the present work. In C. gapperi (Fig. 9), the major part of the tissue consisted of three paired deposits between the scapulae: interscapular, dorsal cervical and subscapular. The volume of tissue was recorded separately for those three deposits but values for the remaining deposits were grouped and treated as a

unit. No deposit was found around the dorsal aorta, and the part of the abdomen anterior to the umbilical cord was devoid of BAT. The volume of BAT if any, posterior to the umbilical cord was assumed to be negligible. No white adipose tissue was observed.

The three deposits between the scapulae constituted about 60% of the volume of all deposits and the interscapular deposit _per se_, approximately 25%. The correlation between any of those three values and the total volume was always high ($P < 0.001$), indicating that the volume of interscapular BAT in _C. gapperi_ embryos is a good index of the total volume. It seems unlikely that this relationship would disappear if mass instead of volume was considered. There was no significant difference between the volumes of BAT present in each side of the embryos.

Figure 11 suggests that the relative volume of BAT in embryos is proportional to their body mass. This relationship was present within each pair of embryos, where variables such as litter size and maternal BAT were constant. According to the relative mass of their interscapular BAT, mothers could be grouped in two categories, one comprising animals _e_, _f_ and _b_ (.20 - .21%) and the other, animals _a_, _c_ and _d_ (.24 - .25%) (Table 3). If relative amount of embryonic BAT is dependent on relative amount of maternal BAT, the former value should be lower in embryos from the first group when compared to embryos from similar litter sizes in the second but this was not so (Fig. 11). If relative amount of BAT in embryos depends on the size of the

litter they come from, it should be higher in embryos d (litter size = 2) than in embryos a and e (litter size = 5) and embryos b, c and f (litter size = 7) but it is not (Fig. 11). The regression lines of relative volume of BAT of embryos on their body mass, even though embryos came from different periods, did not differ greatly in the two trapping seasons (Fig. 11).

Peromyscus maniculatus

Wild population

Correlation coefficients between body mass of animals on one hand and their absolute and relative mass of interscapular BAT on the other were plotted (Fig. 12). If the mass of BAT is proportional to body size, the former coefficients should be positive ($r_A > 0$) and the latter close to zero ($r_R \approx 0$) but this was seldom observed. In May 1967, when populations consisted exclusively of overwintered animals (Fuller, 1969), both coefficients were strongly positive in males and negative in females. This may be related to the significant difference in body mass between the two sexes that also occurred during that month (Fuller, op. cit.). From the second part of June in the two years, when the population was composed of both juveniles and adults, r_A coefficients were generally positive and r_R coefficients negative in both sexes. Thus, if body mass is an index of age, the older the animal the higher the absolute amount of interscapular BAT was likely to be while the reverse was true of the relative amount of that tissue. There was no apparent

reason for the low value of both coefficients in males during late June 1966 or for the decrease in r_A coefficients in females during early August 1966 and in males during early July 1967.

As with C. gapperi, population trends based on live-trapping on permanent plots (Fuller, 1969) did not agree with those based on snap-trapping. Live-trapping showed a steady increase in population numbers in 1966 instead of the irregular fluctuations exhibited by snap-trapping (Fig. 13). Both methods disclosed a decline in 1967, and live-trapping showed that the decline continued through August.

There were clear trends in relative mass of interscapular BAT among females; a significant increase took place during 1966 and a significant decrease in 1967 (Fig. 13; Table 2). In males, the 1966 increase was significant but no definite trend could be detected in 1967. Note that there was no significant difference between early May 1967 and late August 1966 in either sex. Also, individual P. maniculatus always had more BAT than individuals of C. gapperi and there was less fluctuation in the amount present. Three significant differences only were present between values for P. maniculatus (Table 2) as compared with ten in C. gapperi (Table 1).

During 1966, correlation coefficients between trapping success and relative mass of interscapular BAT were significantly different from zero in both sexes (Fig. 14). This was not the

case during the 1967 trapping season in either sex.

The period 15 June to 31 July was trapped in both years and the mean relative mass of interscapular BAT was lower in 1967 (Fig. 15), the difference being significant in females ($t = 2.17$; $P < 0.05$). This situation was paralleled by the lower population in 1967 during that period (Fig. 15) and might have been a predisposing factor to the August 1967 decline in population number mentioned previously.

Food experiment

Following capture, the mean body mass of animals increased in all groups (Fig. 16). Sealander (1951) reports a similar increase at the beginning of captivity for P. noveboracensis. Northern small mammals have an exceptional capacity for storing fat and readily do so in captivity (Schwartz, 1963). In December, in spite of the shift to the bulkier winter diets, mass began to increase gradually. From January to May, the mean body mass of animals in group 1 that received a normal diet throughout the experiment was expected to be significantly higher than that of the others. Group 1 animals were exceeded in mass by those in group 4 and were not significantly heavier than those in groups 2, 3 and 5. After a peak in late April body mass began to decline and the decline was not arrested by a return to a higher quality diet. The rank order at the end of the experiment was the same as at the beginning except that groups 5 and 2 had changed positions. There is thus no evidence that the range of food

quality used had any effect on body mass of the captive animals. Other studies show that food also has little effect on spring changes of body mass in wild populations of voles (Chitty, Pimentel and Krebs, 1968).

Food had some effect however on the relative amount of interscapular BAT. Because of the difference in their diets, animals of group 5 were expected to have the lowest relative as well as absolute mass of interscapular BAT and animals of group 1 the highest of all groups. Those expectations were not fulfilled in the first case but were partially fulfilled in the second. The absolute and relative amounts of BAT in animals of group 1 were significantly greater than that of animals in groups 3 and 4 ($P < 0.05$) (Table 4). Note also that while wild P. maniculatus had a relative mass of interscapular BAT ranging approximately from .20 to .45% of body mass (Fig. 13), the relative mass of that tissue was much higher after 10 months of captivity and ranged from .74 to 1.07% of body mass. Although none of the animals had shown perforation of the vagina, ovulation had taken place in many animals including some of group 5.

DISCUSSION

Because of lack of data for the 1966 spring critical period, it is impossible to interpret the values of interscapular BAT that accompanied the disappearance of animals during the same

period in 1967. During the 1966 population increase that followed the critical period, positive correlations, although based on small samples, seem to have been present in both species between population numbers and relative mass of interscapular BAT. These correlations appear to have been the result of the simultaneous contribution of the juveniles to both the increase in population number and the increase in relative mass of interscapular BAT.

During the 1967 population decrease no correlation existed except for female C. gapperi. More than one interpretation could fit that situation. First, a lack of relation between the amount of BAT and survival. However, that explanation does not account for the high value of the correlation in female C. gapperi. Second, the presence of low population numbers in periods when relative mass of interscapular BAT was high. In both species, relative mass of interscapular BAT was probably not if at all, the only factor limiting the population; consequently, low population numbers could be expected in periods in which relative amounts of BAT were high. Correlation coefficients used to measure such a "unidirectional" relationship, meaningful when close to significance, are difficult to interpret when not. Third, a biased sample. Any sample of animals taken after a decline in population number resulting from mortality is biased in the sense that it is uniquely composed of the survivors. If the amount of interscapular BAT was involved in animal mortality, its values, measured in captured animals, came from those that possessed enough of it to stay alive.

Absence of trends in relative mass of interscapular BAT while the population of C. gapperi was declining, significantly reduced values of that tissue in female P. maniculatus preceeding a decline in population number and, in a study of M. pennsylvanicus (Didow, 1968), relative mass of BAT much lower in April 1967, when population was low than in the same month in 1966, a year of peak population, seem to suggest that low relative mass of interscapular BAT is involved in population limitation. However despite this suggestive evidence, the present data do not confirm the existence of a relationship between relative mass of interscapular BAT and survival of adults in summer. A study of BAT, if the circumstances had permitted, during a series of spring and fall critical periods could have been more profitable.

Winter activity of P. maniculatus is reduced as compared with that of C. gapperi (Stebbins, 1968). The two species are also obviously equipped to eat different food. Thus it is not surprising that BAT decreased over winter in C. gapperi but not in P. maniculatus. Another interesting feature appears in the comparison of the interscapular BAT in the two species: P. maniculatus having more exposed body parts (ears and tail) and a more slender body than C. gapperi also has a higher relative mass of interscapular BAT.

The absence of major differences in amounts of embryonic BAT between the two years suggests that, at this level, BAT was not involved in population limitation. However, the increase

in relative mass of BAT in C. gapperi embryos as they increased in size followed by the negative correlation between the relative level of that tissue and body size in the months when juveniles were present in the population, indicate that the peak relative amounts of BAT occurred sometime between birth of the animals and the time when they started to appear in the traps (5 weeks). In lemmings, the highest relative mass of interscapular BAT occurs at birth when it constitutes approximately .8% of body mass (Hissa, 1968). In rabbits, the highest relative amounts of BAT also occur in newborn (Dawkins and Hull, 1964).

Probably none of the captive P. maniculatus, regardless of their diet, suffered any energetic deficiency as can be concluded from the presence of fat deposits around the ovaries of all animals, from their relatively high interscapular BAT as compared with that of wild animals, from the absence of any obvious differences between mean body mass of any of the five groups and finally from ovulation having taken place in all groups. Food does not seem to have been involved in the shifts that took place in body mass suggesting in natural populations of P. maniculatus the possibility of similar shifts not resulting from food. But because the mass of interscapular BAT in animals that received the "normal" diet was significantly higher than that of animals of two of the other groups, quality of the diet may influence the amount of BAT present in wild animals in winter. However, if it limits it, it probably involves dietary inadequacy of a higher degree or of a different nature than those tested in the present study.

TABLE 1

Summary of semimonthly parameters concerning relative mass of interscapular brown adipose tissue (BAT) for *Clethrionomys gapperi*

Sex	Date			Means	Significant differences between means*	Sample size	Standard deviation	Minimum	Maximum	Range
FEMALE	1966	June	1-15	-	-	-	-	-	-	-
			16-30	.25	a1	7	.14	.07	.41	.34
		July	1-15	.21	a1	2	.11	.13	.29	.16
			16-31	.30	a	11	.11	.19	.49	.30
		August	1-15	.29	a1	17	.09	.17	.46	.29
			16-31	.42	a2-b1	5	.09	.28	.52	.24
	1967	May	1-15	.23	c -b2	10	.05	.19	.35	.16
			16-31	.27	c1--g1	11	.04	.22	.34	.12
		June	1-15	.23	c2	10	.04	.18	.32	.14
			16-30	.31	c	1	-	-	-	-
		July	1-15	.29	c	7	.16	.12	.52	.40
			16-31	.33	c	3	.22	.11	.32	.21
MALE	1966	June	1-15	-	-	-	-	-	-	-
			16-30	.24	d1	8	.07	.11	.32	.21
		July	1-15	.16	d1	3	.04	.12	.20	.08
			16-31	.30	d	23	.14	.13	.70	.57
		August	1-15	.34	d2	16	.09	.23	.51	.28
			16-31	.35	d -e1	5	.14	.20	.52	.32
	1967	May	1-15	.25	f1-e2	9	.04	.16	.31	.15
			16-31	.19	f2-g2	21	.06	.12	.33	.21
		June	1-15	.25	f	5	.09	.13	.38	.25
			16-30	.23	f	2	.01	.23	.23	.00
		July	1-15	.21	f	1	-	-	-	-
			16-31	.16	f	2	.11	.08	.24	.16

*Means with same letter were compared; a1 differed significantly from a2, b1 from b2, etc.

TABLE 2

Summary of semimonthly parameters concerning relative mass of interscapular brown adipose tissue (BAT) for <u>Peromyscus maniculatus</u>

Sex	Date			Means	Significant differences between means*	Sample size	Standard deviation	Minimum	Maximum	Range
FEMALE	1966	June	1-15	-	-	-	-	-	-	-
			16-30	.27	a1	2	.05	.23	.31	.08
		July	1-15	.32	a	4	.07	.25	.42	.17
			16-31	.42	a	27	.15	.27	1.01	.74
		August	1-15	.42	a	6	.14	.26	.66	.40
			16-31	.45	a2	11	.11	.25	.64	.39
	1967	May	1-15	.43	b	3	.07	.35	.49	.14
			16-31	.45	b1	4	.12	.36	.61	.25
		June	1-15	.35	b	15	.09	.16	.48	.32
			16-30	.35	b	5	.06	.28	.44	.16
		July	1-15	.27	b2	6	.11	.11	.44	.33
			16-31	.20	b	1	-	-	-	-
MALE	1966	June	1-15	-	-	-	-	-	-	-
			16-30	.34	c1	5	.07	.27	.46	.19
		July	1-15	.43	c	7	.13	.27	.64	.37
			16-31	.42	c	22	.10	.23	.67	.44
		August	1-15	.42	c	13	.07	.27	.50	.33
			16-31	.44	c2	9	.07	.34	.56	.22
	1967	May	1-15	.44	d	6	.14	.29	.70	.41
			16-31	.39	d	4	.08	.29	.49	.20
		June	1-15	.34	d	14	.11	.24	.62	.38
			16-30	.35	d	2	.03	.32	.37	.05
		July	1-15	.35	d	5	.17	.12	.53	.41
			16-31	.40	d	7	.11	.24	.57	.33

*Means with same letter were compared; a1 differed significantly from a2, b1 from b2, etc.

TABLE 3

Relative amounts (volume and mass) of interscapular brown adipose tissue (BAT) in <u>Clethrionomys gapperi</u> embryos and in their mothers from both the 1966 and 1967 trapping seasons

	Date of capture	Mother	Relative mass of interscapular BAT in mothers (% of body mass)	Litter size	Location of embryo in uterus*	Mass of embryos (g)	Relative volume of BAT in embryos (% of body volume)
1966	July 28	a	.24	5	L4	1.76	.73
					R1	1.95	.75
	August 1	b	.21	7	L3	1.16	.48
					R2	1.11	.46
	August 2	c	.25	5	L2	1.48	.46
					R2	1.36	.42
1967	June 7	d	.24	2	R1	2.07	.77
					R2	1.54	.42
	June 7	e	.20	5	L2	1.82	.73
					R2	2.05	.84
	July 8	f	.21	7	L1	1.04	.39
					R3	0.96	.26

*L and R refer to left and right cornua, numbers to rank order of embryos counting from vagina

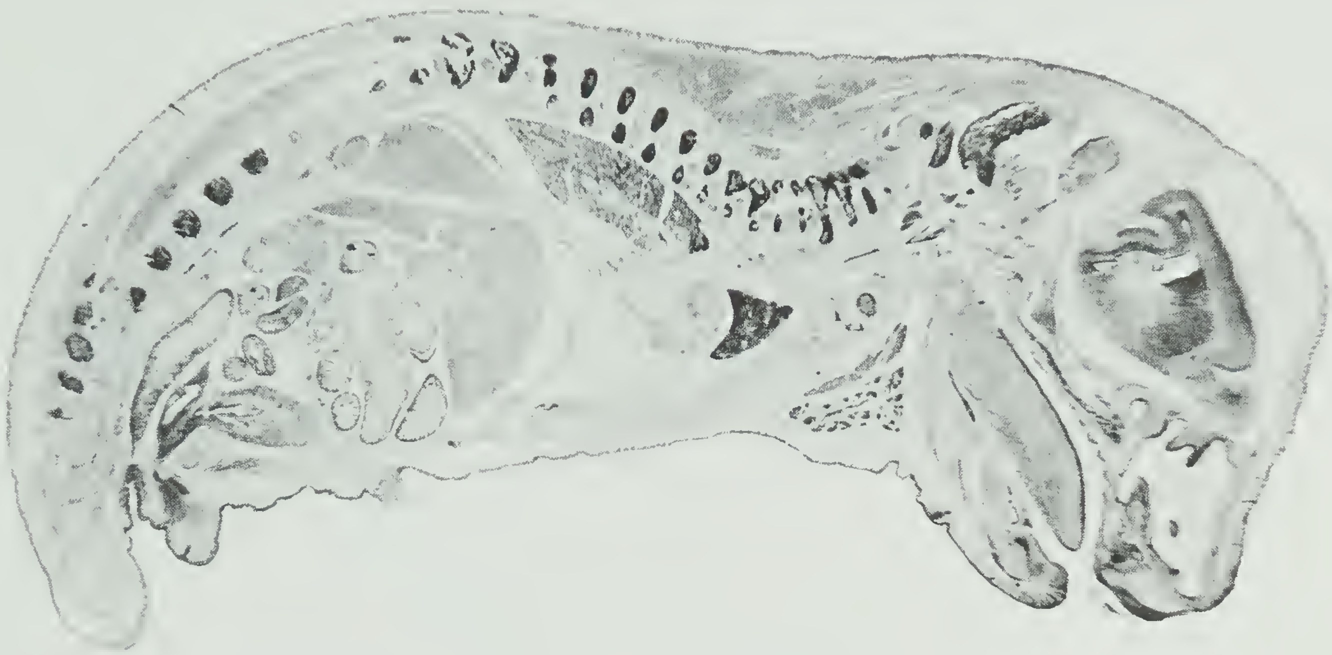
TABLE 4

Winter and spring diets served to captive *Peromyscus maniculatus* and amounts (absolute and relative mass) of interscapular brown adipose tissue (BAT) in animals at the end of the experiment

Group number	Sample size	DIET		Mean mass of interscapular brown adipose tissue (BAT)	
		Winter	Spring	absolute (g)	relative (% of body mass)
1	4	normal	normal	.23	1.07
2	4	bulky	normal	.16	.82
3	6	bulky	normal + sprouted wheat	.14	.74
4	6	bulky	bulky + sprouted wheat	.18	.84
5	6	bulky	bulky	.19	.92

FIGURE 1

Photograph of sagittal section of laboratory mouse (*Mus musculus*) embryo showing distribution of major deposits of brown adipose tissue (BAT). $\times 6$.



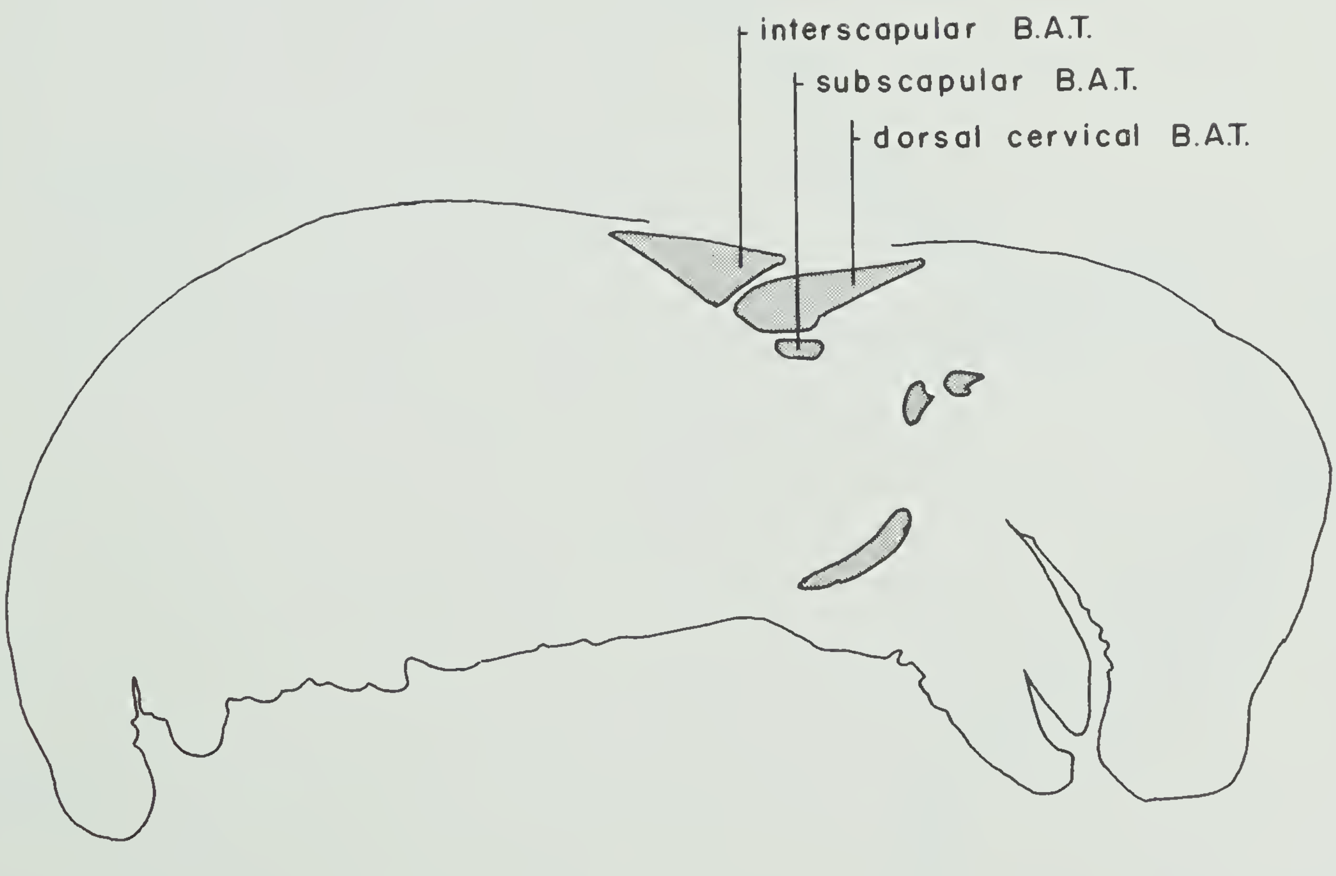

interscapular B.A.T.
subscapular B.A.T.
dorsal cervical B.A.T.

FIGURE 2

Map of the study area showing territory where snap- and live-trapping were carried out.

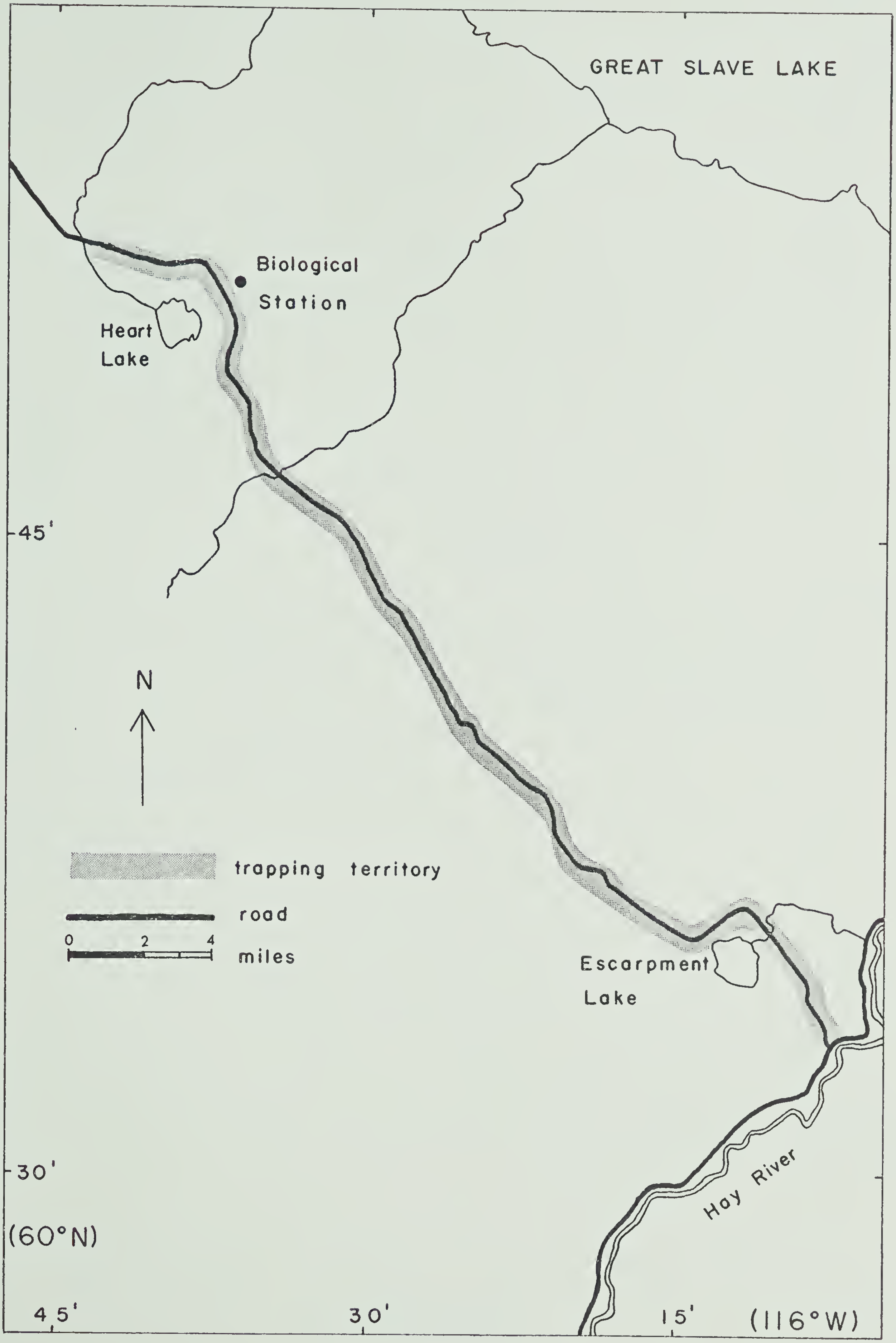

GREAT SLAVE LAKE
Biological
Station
Heart
Lake
45'
N
trapping territory
road
0
2
4
miles
Escarpment
Lake
30'
(60°N)
Hay River
45'
30'
15'
(116°W)

FIGURE 3

Mean monthly temperatures at Hay River Airport during 1966 and 1967.

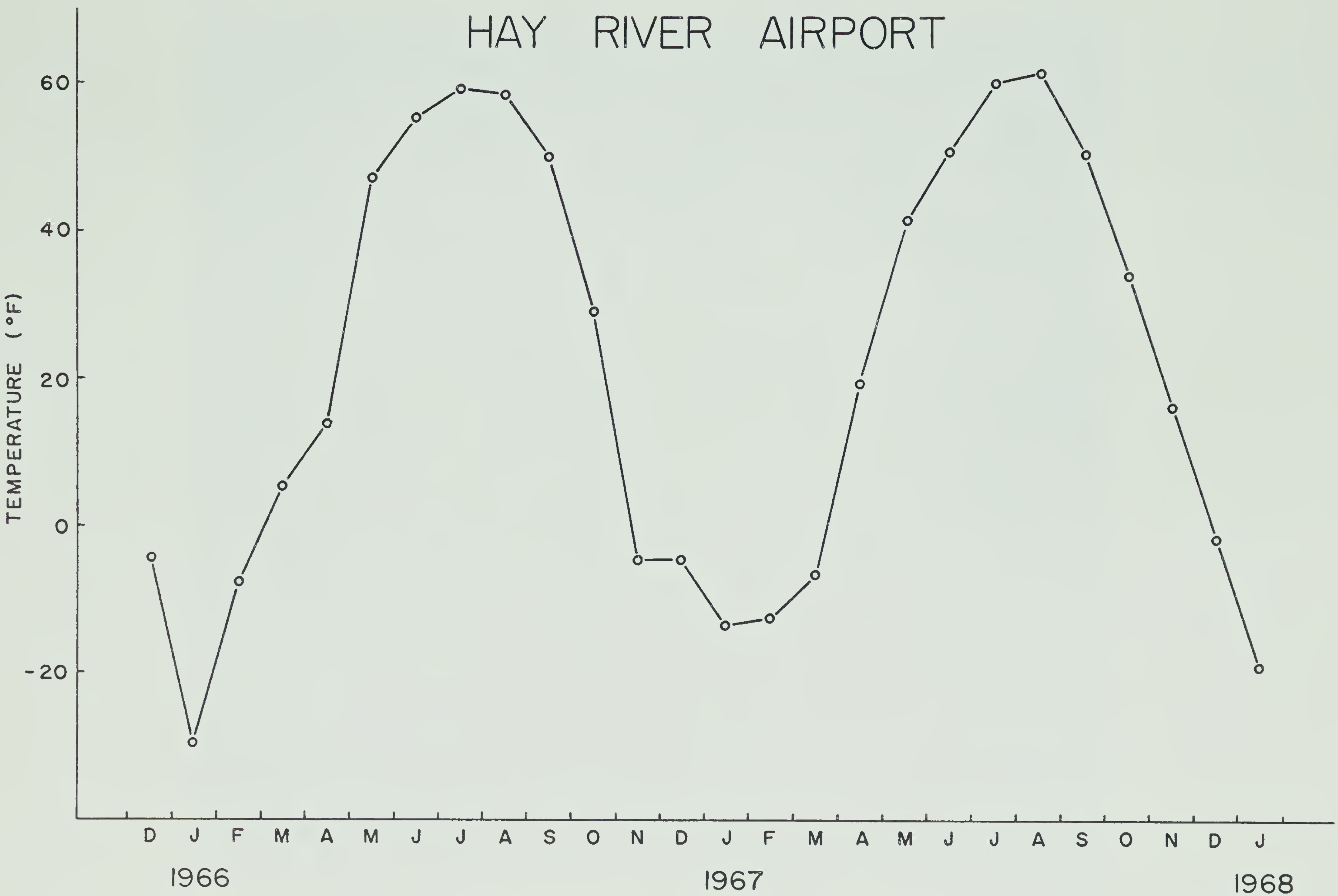

HAY RIVER AIRPORT
TEMPERATURE (°F)
60
40
20
0
-20
D J F M A M J J A S O N D J F M A M J J A S O N D J
1966
1967
1968

FIGURE 4

Photograph of Clethrionomys gapperi embryo. Shaded areas show parts removed to insure adequate infiltration during histological processing. ×5.

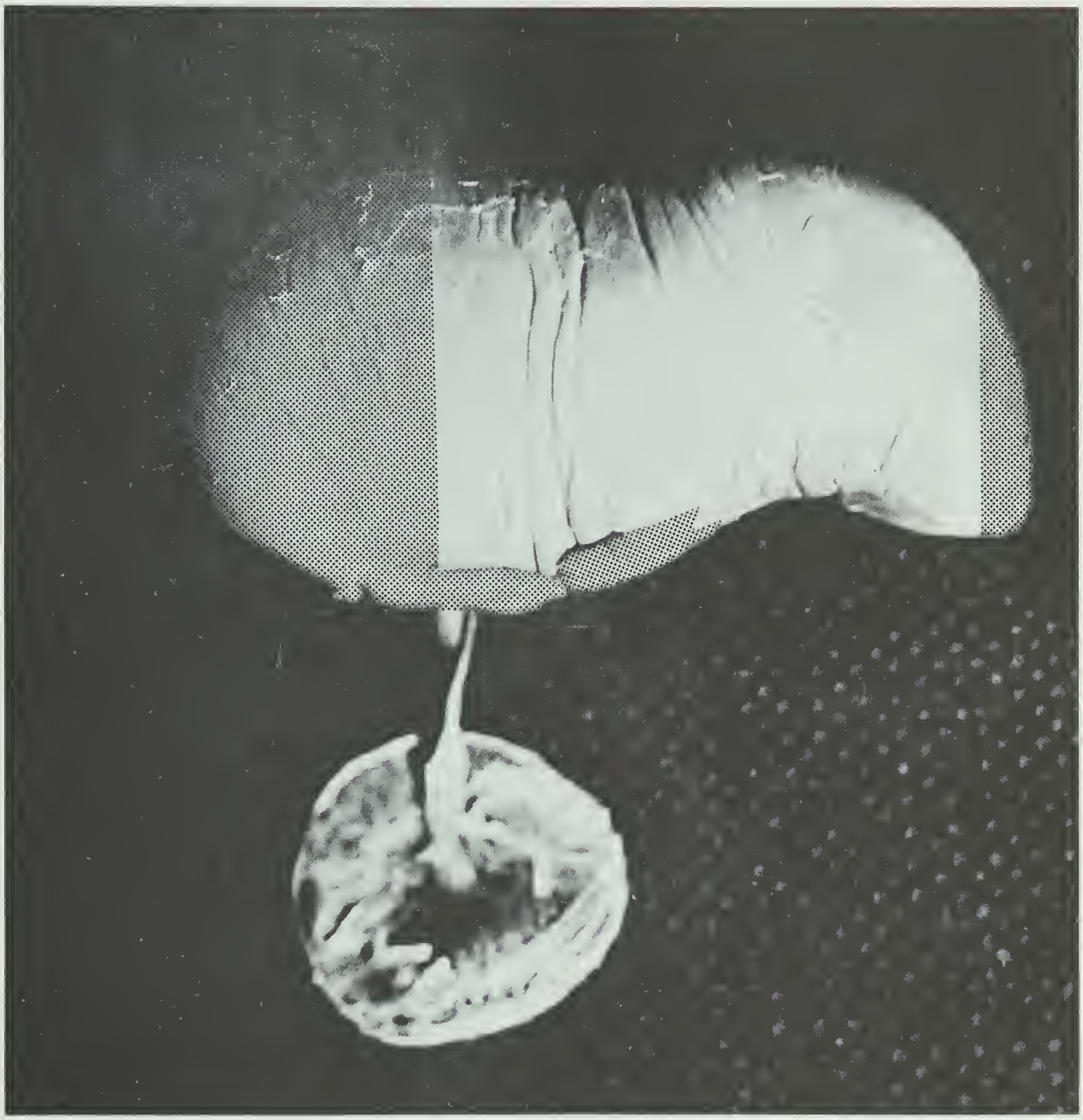


FIGURE 5

Photograph of racks and cages used for *Peromyscus maniculatus*. The photograph was taken after the experiment was over and there were no animals nor feeders inside the cages.

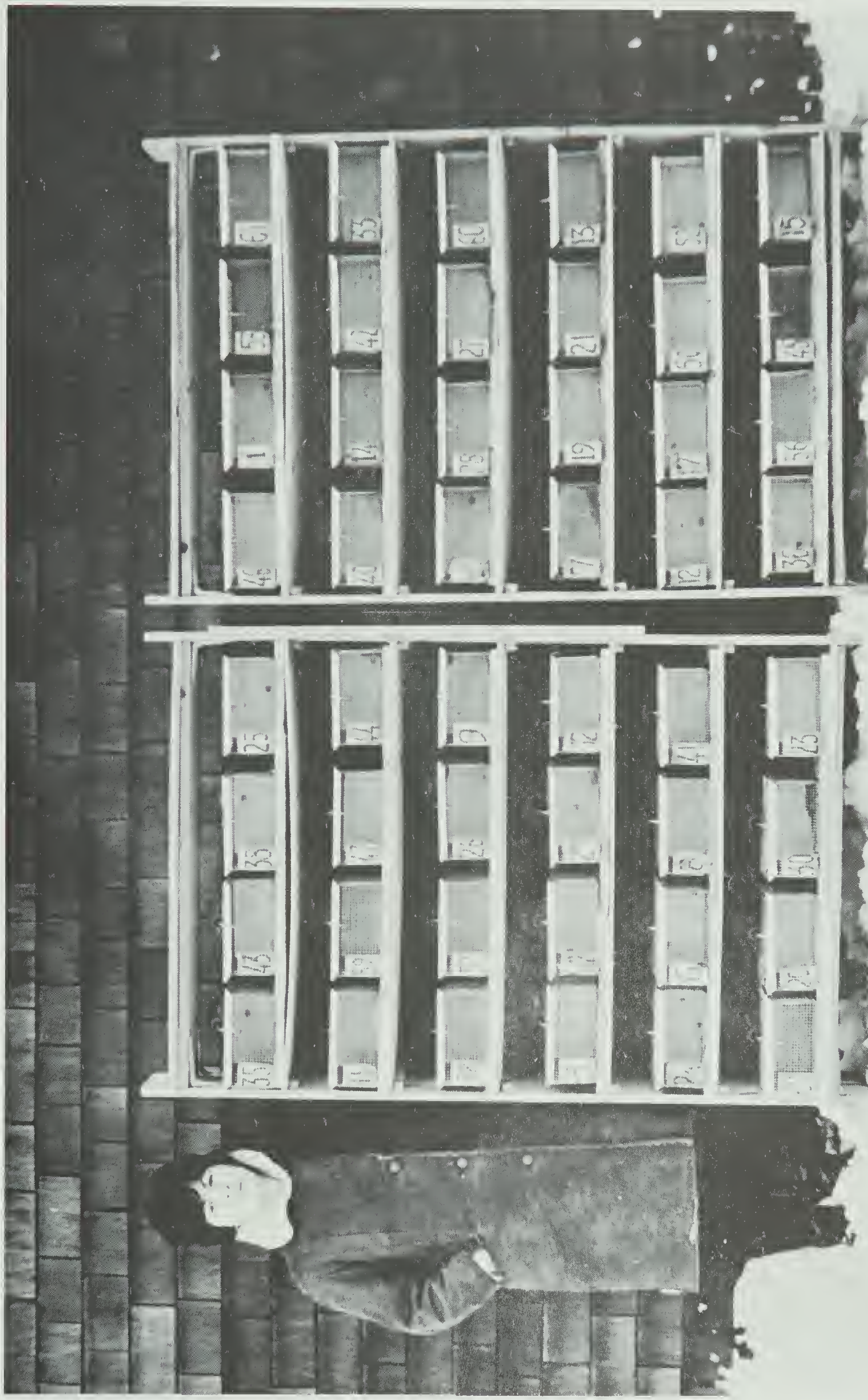
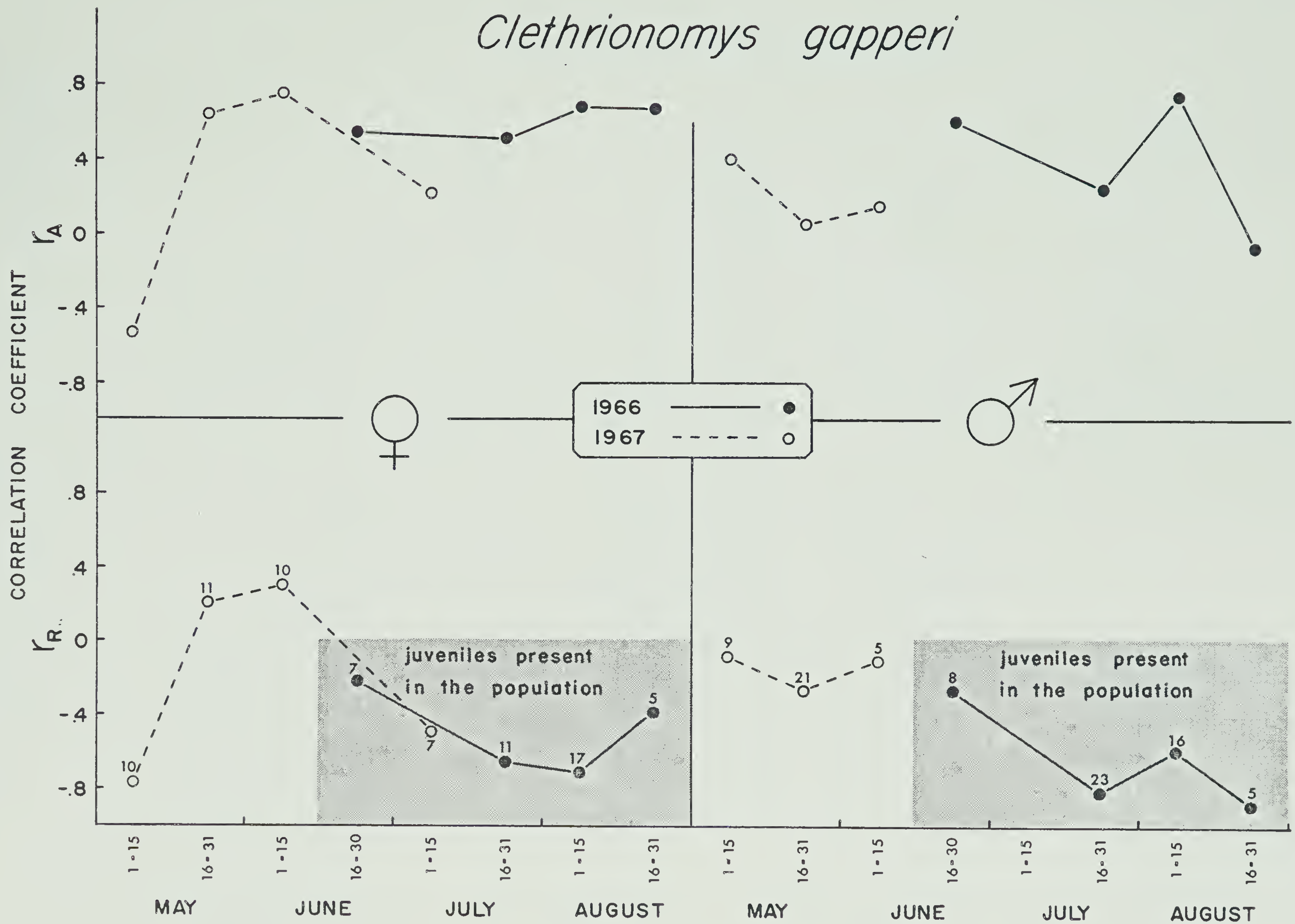


FIGURE 6

Correlation coefficients (r) between mass [absolute (A) and relative (R)] of interscapular brown adipose tissue (BAT) and body mass for *Clethrionomys gapperi* in trapping periods when more than three animals were caught. Figures show number of animals in sample.



Clethrionomys gapperi
CORRELATION COEFFICIENT
r_A
r_R
.8
.4
0
-.4
-.8
1966
1967
juveniles present in the population
1-15
16-31
16-30
MAY
JUNE
JULY
AUGUST

FIGURE 7

Fluctuations in trapping success and in relative mass of interscapular brown adipose tissue (BAT) for *Clethrionomys gapperi*. Figures above bars show number of animals in sample.

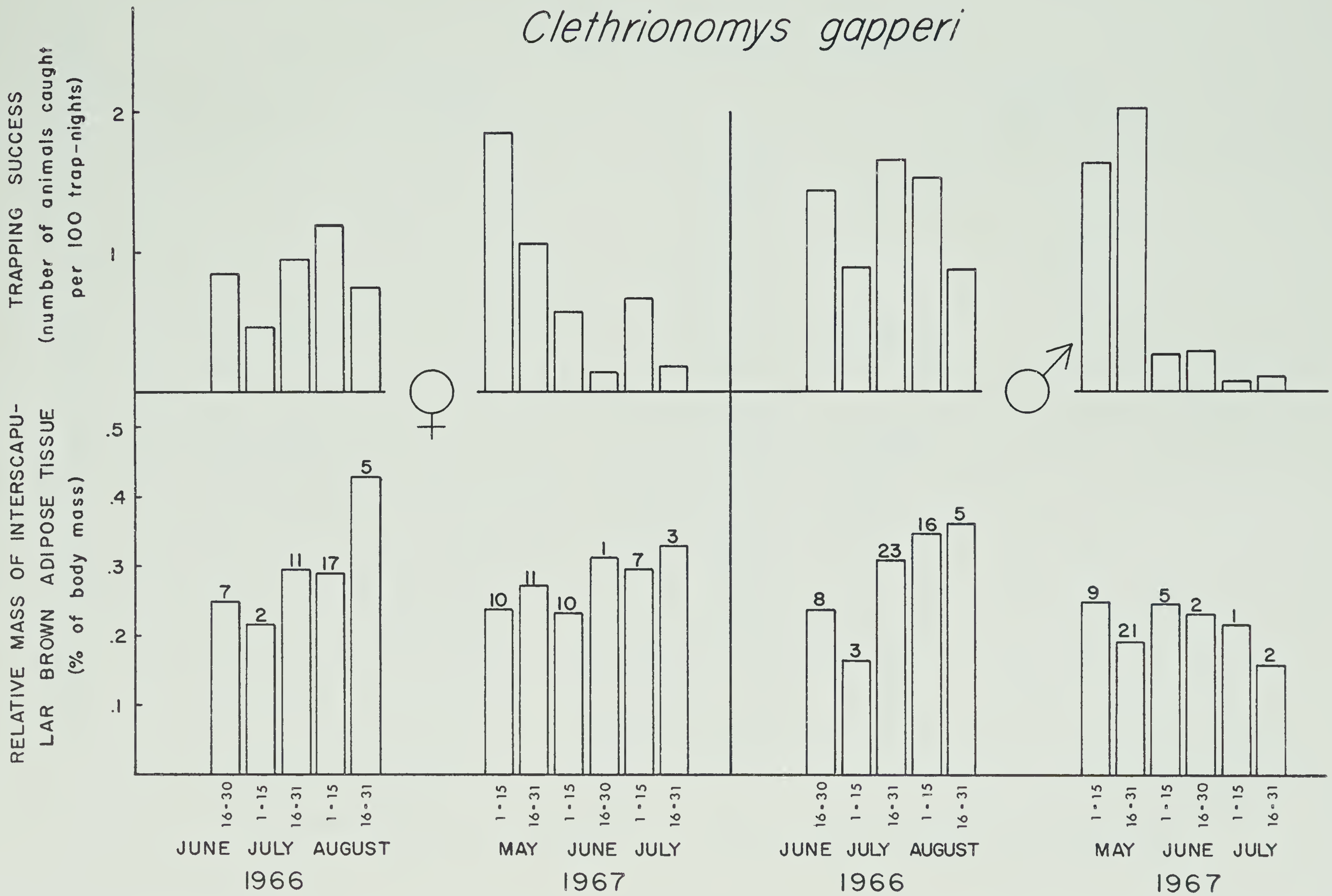

Clethrionomys gapperi
TRAPPING SUCCESS
(number of animals caught
per 100 trap-nights)
2
1
RELATIVE MASS OF INTERSCAPU-
LAR BROWN ADIPOSE TISSUE
(% of body mass)
.5
.4
.3
.2
.1
♀
♂
7
2
11
17
5
10
11
10
1
7
3
8
3
23
16
5
9
21
5
2
1
2
16-30
1-15
16-31
1-15
16-31
1-15
16-31
1-15
16-30
1-15
16-31
16-30
1-15
16-31
1-15
16-31
1-15
16-31
1-15
16-30
1-15
16-31
JUNE JULY AUGUST
1966
MAY JUNE JULY
1967
JUNE JULY AUGUST
1966
MAY JUNE JULY
1967

FIGURE 8

Relationship between trapping success and relative mass of interscapular brown adipose tissue (BAT) for *Clethrionomys gapperi* and correlation coefficients between same variates.

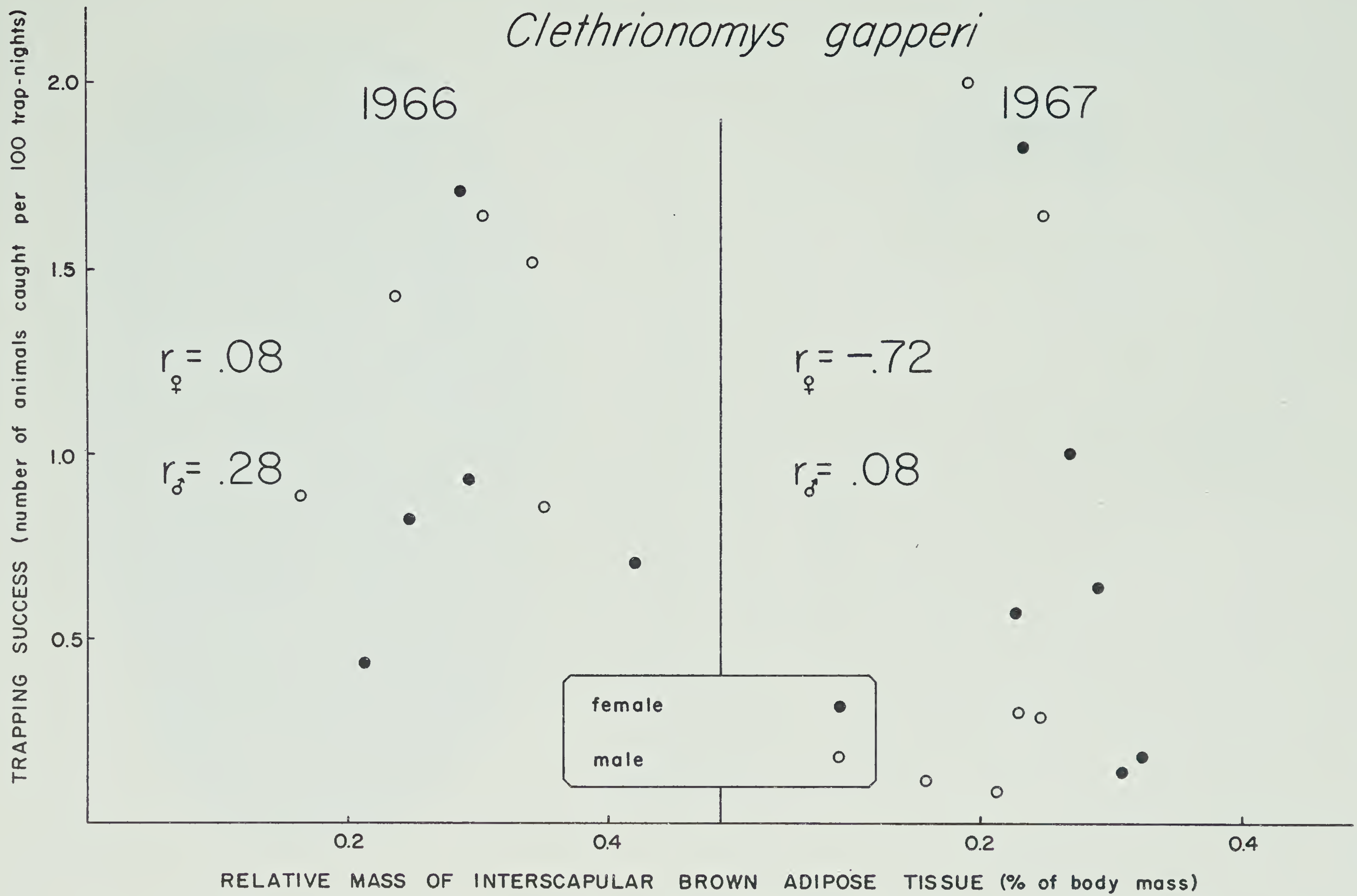
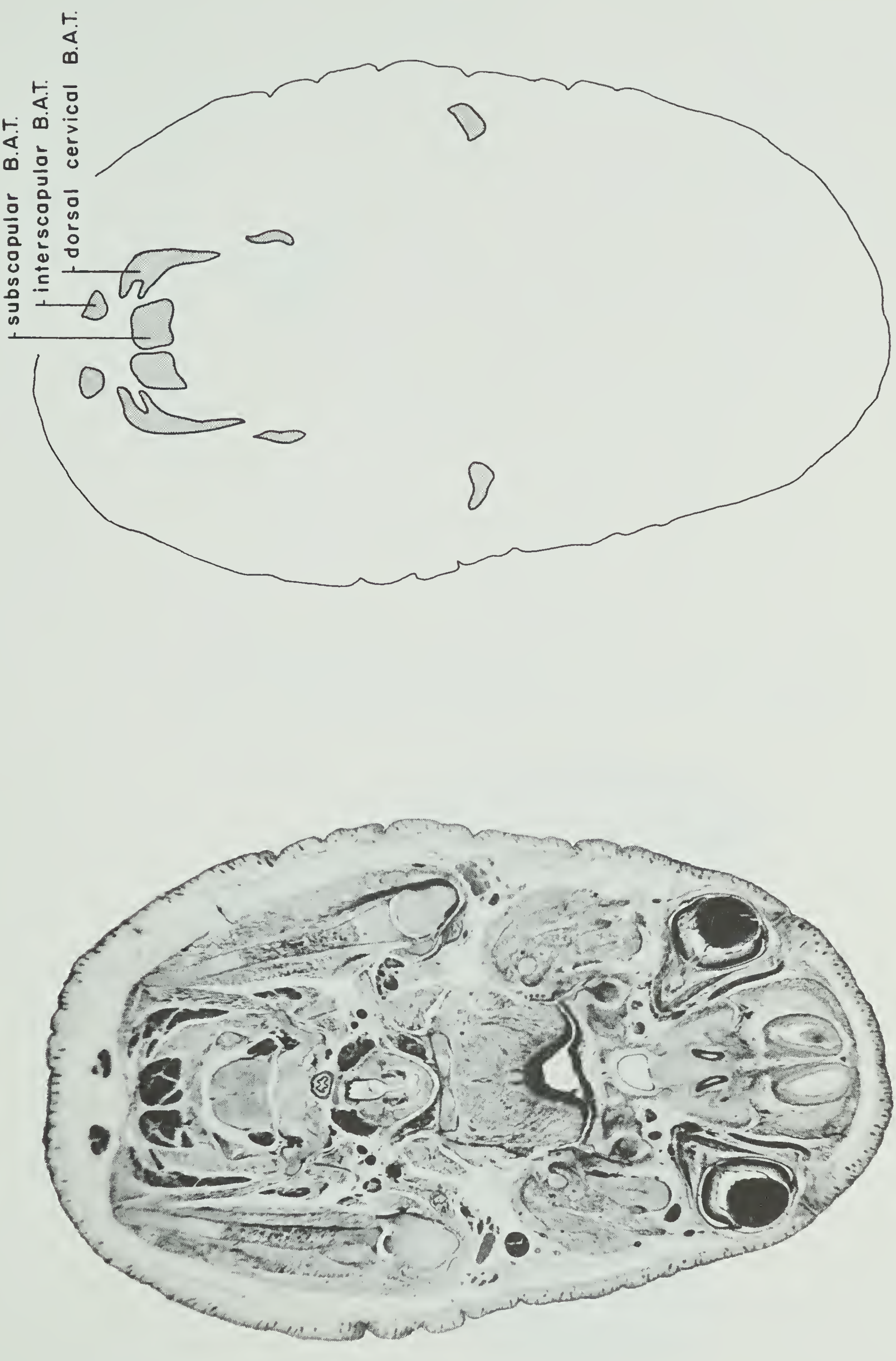

Clethrionomys gapperi
1966
1967
r♀= .08
r♂= .28
r♀= −.72
r♂= .08
female
male
2.0
1.5
1.0
0.5
0.2
0.4
0.2
0.4
TRAPPING SUCCESS (number of animals caught per 100 trap-nights)
RELATIVE MASS OF INTERSCAPULAR BROWN ADIPOSE TISSUE (% of body mass)

FIGURE 9

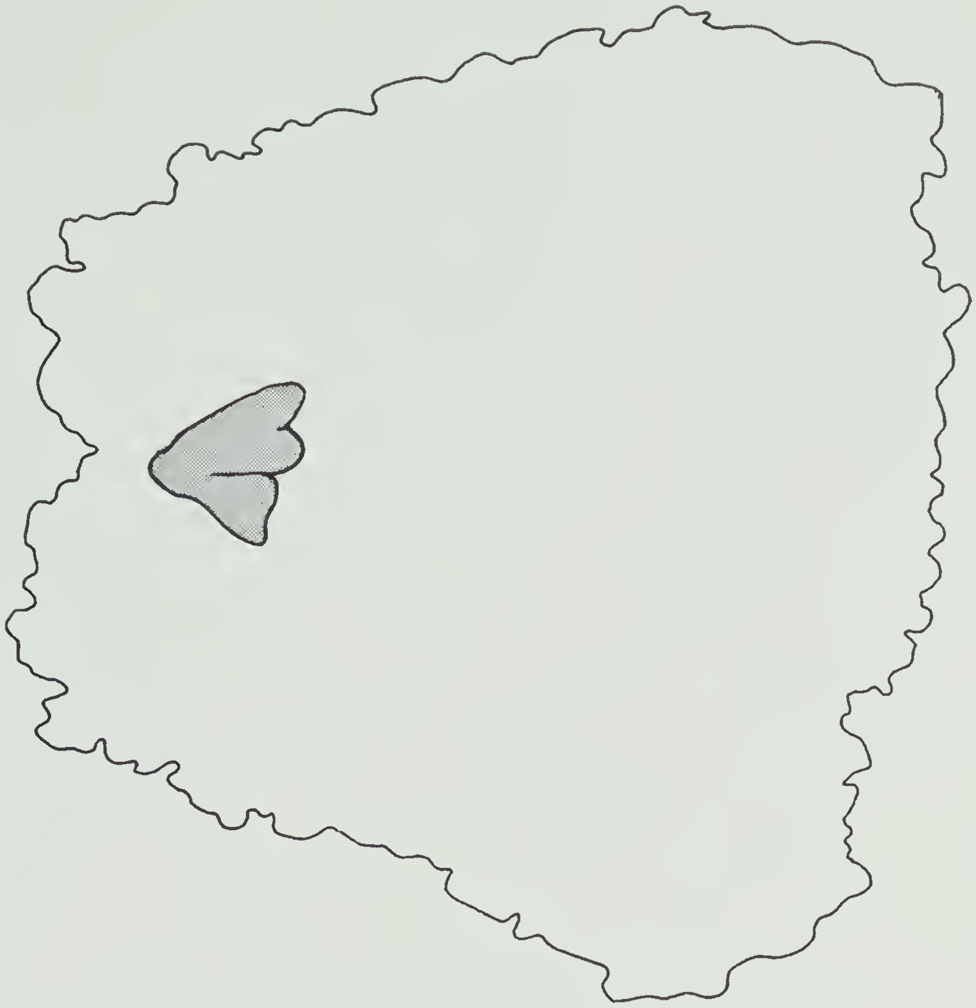
Cross section of <u>Clethrionomys</u> <u>gapperi</u> embryo showing major deposits of brown adipose tissue (BAT) between the scapulae. ×13.



subscapular B.A.T.
interscapular B.A.T.
dorsal cervical B.A.T.

FIGURE 10

Cross section of newborn hamster (*Mesocricetus auratus*) showing interscapular brown body. $\times 13$.



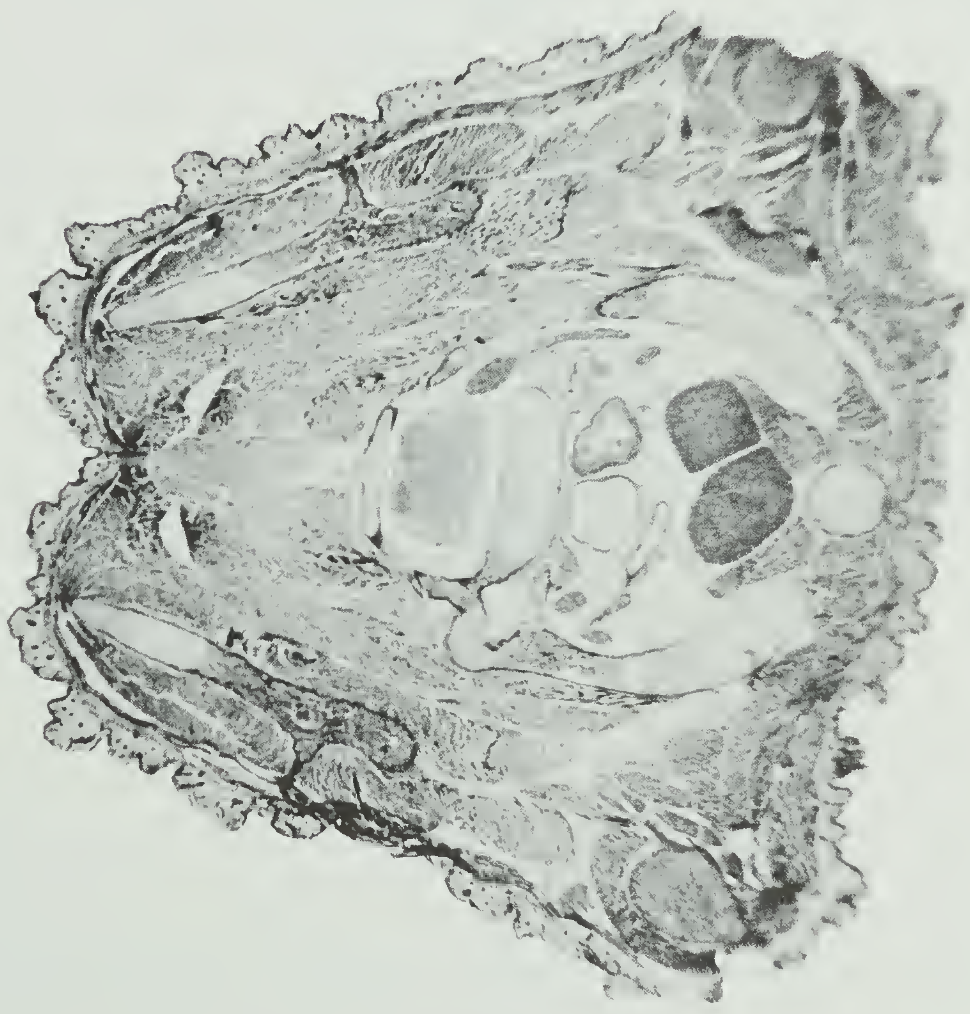


FIGURE 11

Relationship between relative volume of brown adipose tissue (BAT) and body mass for *Clethrionomys gapperi* embryos. *a*-*f*: females as in Table 3. *1*, *2*: individual embryo in litter.

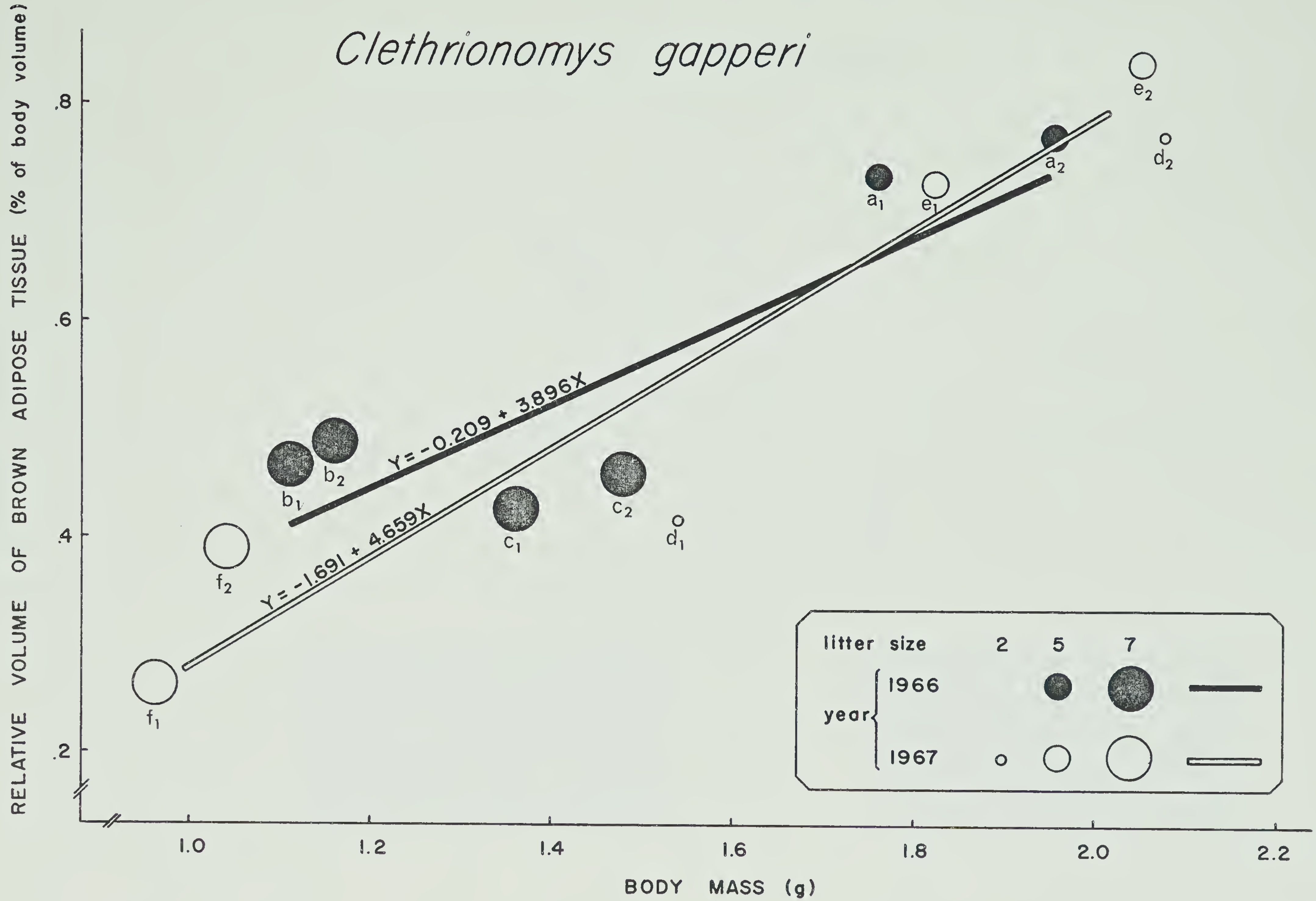
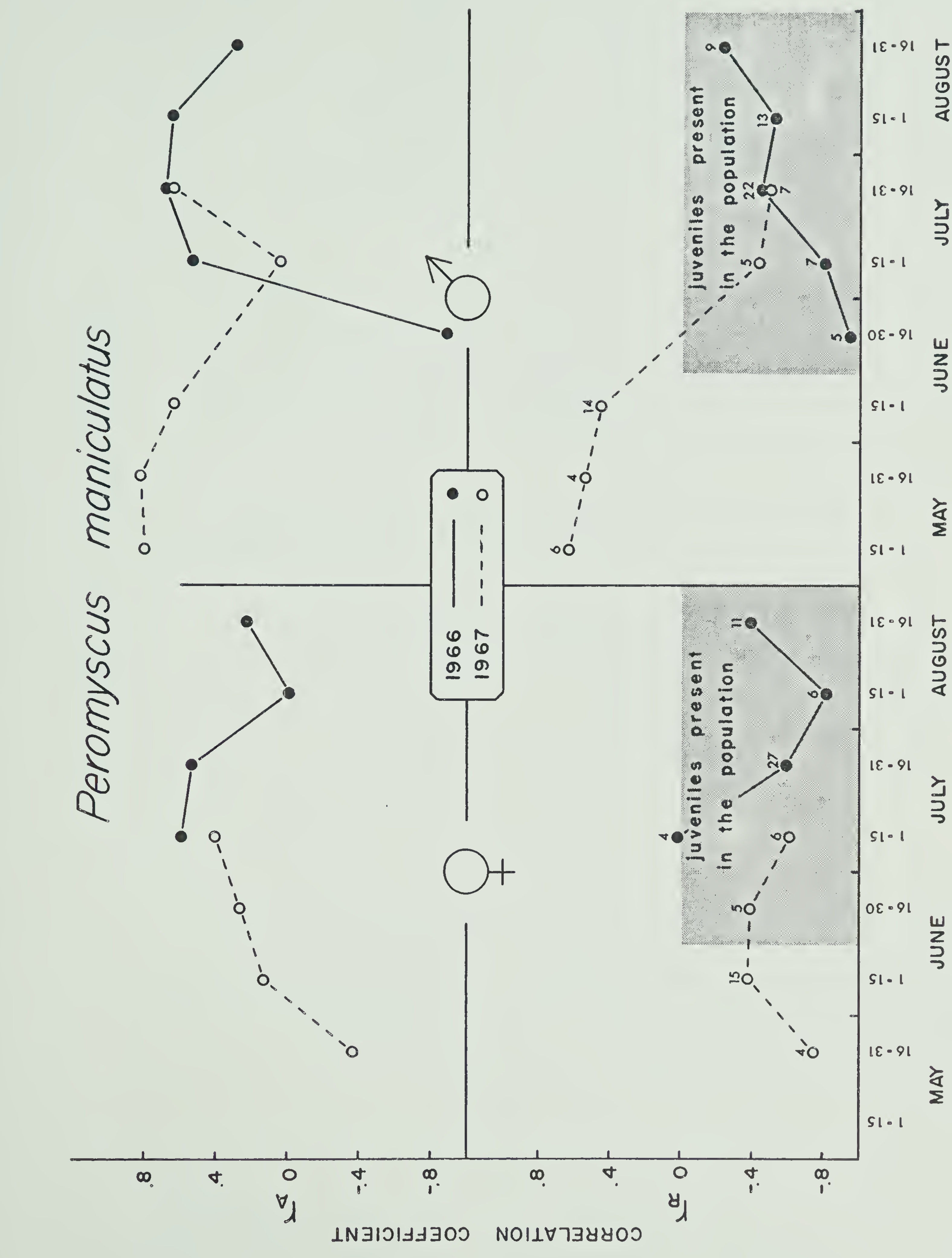

Clethrionomys gapperi
RELATIVE VOLUME OF BROWN ADIPOSE TISSUE (% of body volume)
BODY MASS (g)
Y = -0.209 + 3.896X
Y = -1.691 + 4.659X
litter size 2 5 7
year 1966
1967
a1 a2 b1 b2 c1 c2 d1 d2 e1 e2 f1 f2
.2 .4 .6 .8
1.0 1.2 1.4 1.6 1.8 2.0 2.2

FIGURE 12

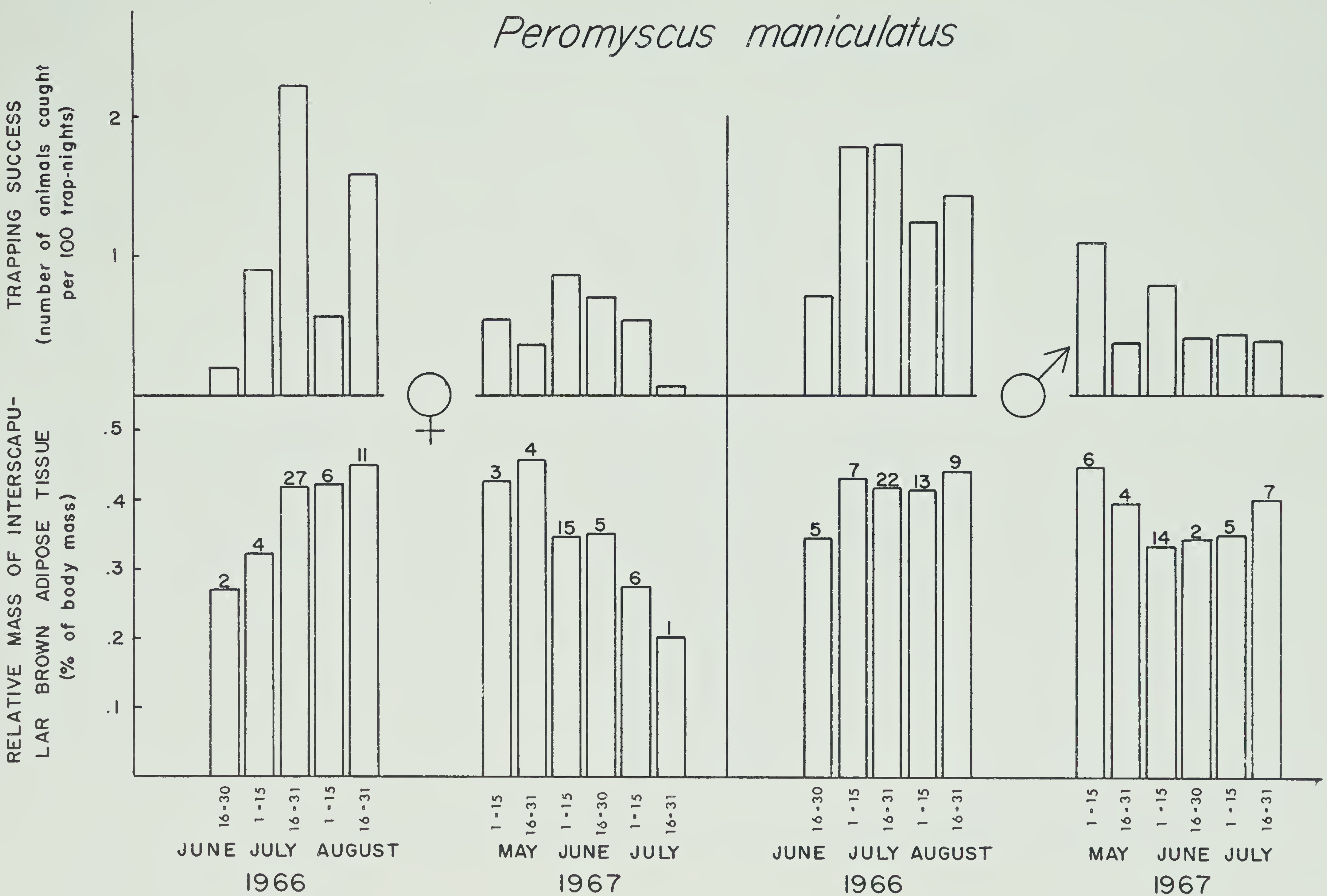
Correlation coefficients (r) between mass [absolute (A) and relative (R)] of interscapular brown adipose tissue (BAT) and body mass for *Peromyscus maniculatus* in trapping periods when more than three animals were caught. Figures show number of animals in sample.



Peromyscus maniculatus
CORRELATION COEFFICIENT
γ_A
γ_R
.8
.4
0
-.4
-.8
1966
1967
juveniles present in the population
juveniles present in the population
1-15
16-31
1-15
16-30
1-15
16-31
1-15
16-31
MAY
JUNE
JULY
AUGUST
MAY
JUNE
JULY
AUGUST

FIGURE 13

Fluctuations in trapping success and in relative mass of interscapular brown adipose tissue (BAT) for Peromyscus maniculatus. Figures above bars show number of animals in sample.



Peromyscus maniculatus
TRAPPING SUCCESS
(number of animals caught
per 100 trap-nights)
RELATIVE MASS OF INTERSCAPU-
LAR BROWN ADIPOSE TISSUE
(% of body mass)
2
1
.5
.4
.3
.2
.1
2
4
27
6
11
3
4
15
5
6
1
5
7
22
13
9
6
4
14
2
5
7
16-30
1-15
16-31
1-15
16-31
JUNE JULY AUGUST
1966
1-15
16-31
1-15
16-30
1-15
16-31
MAY JUNE JULY
1967
16-30
1-15
16-31
1-15
16-31
JUNE JULY AUGUST
1966
1-15
16-31
1-15
16-30
1-15
16-31
MAY JUNE JULY
1967

FIGURE 14

Relationship between trapping success and relative mass of interscapular brown adipose tissue (BAT) for *Peromyscus maniculatus* and correlation coefficients between same variates.

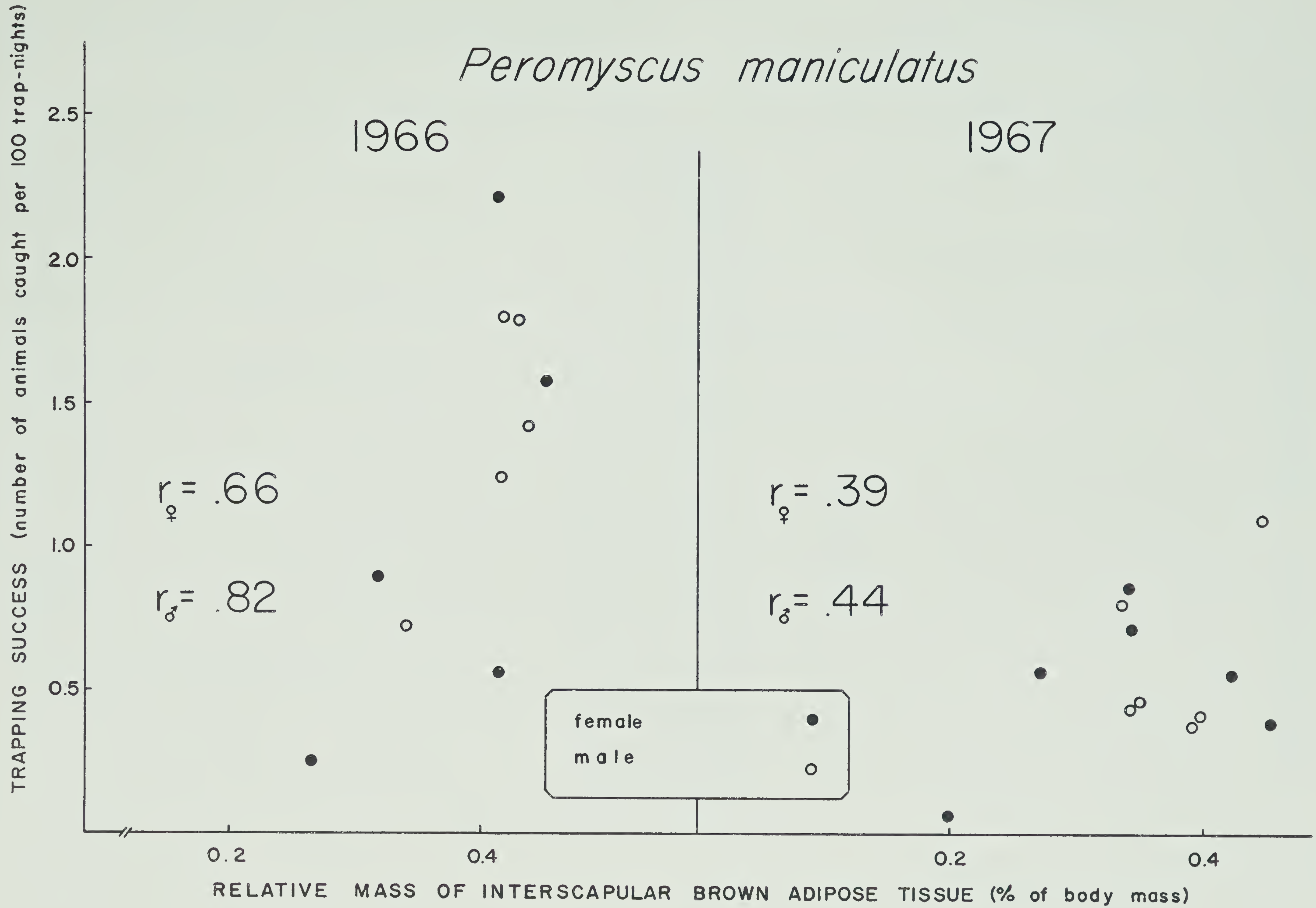

Peromyscus maniculatus
1966
1967
r♀= .66
r♂= .82
r♀= .39
r♂= .44
female
male
TRAPPING SUCCESS (number of animals caught per 100 trap-nights)
2.5
2.0
1.5
1.0
0.5
0.2
0.4
0.2
0.4
RELATIVE MASS OF INTERSCAPULAR BROWN ADIPOSE TISSUE (% of body mass)

FIGURE 15

Trapping success and relative mass of interscapular brown adipose tissue (BAT) for *Peromyscus maniculatus* during late June and July. Symbols show mean, standard deviation, range and number in sample.

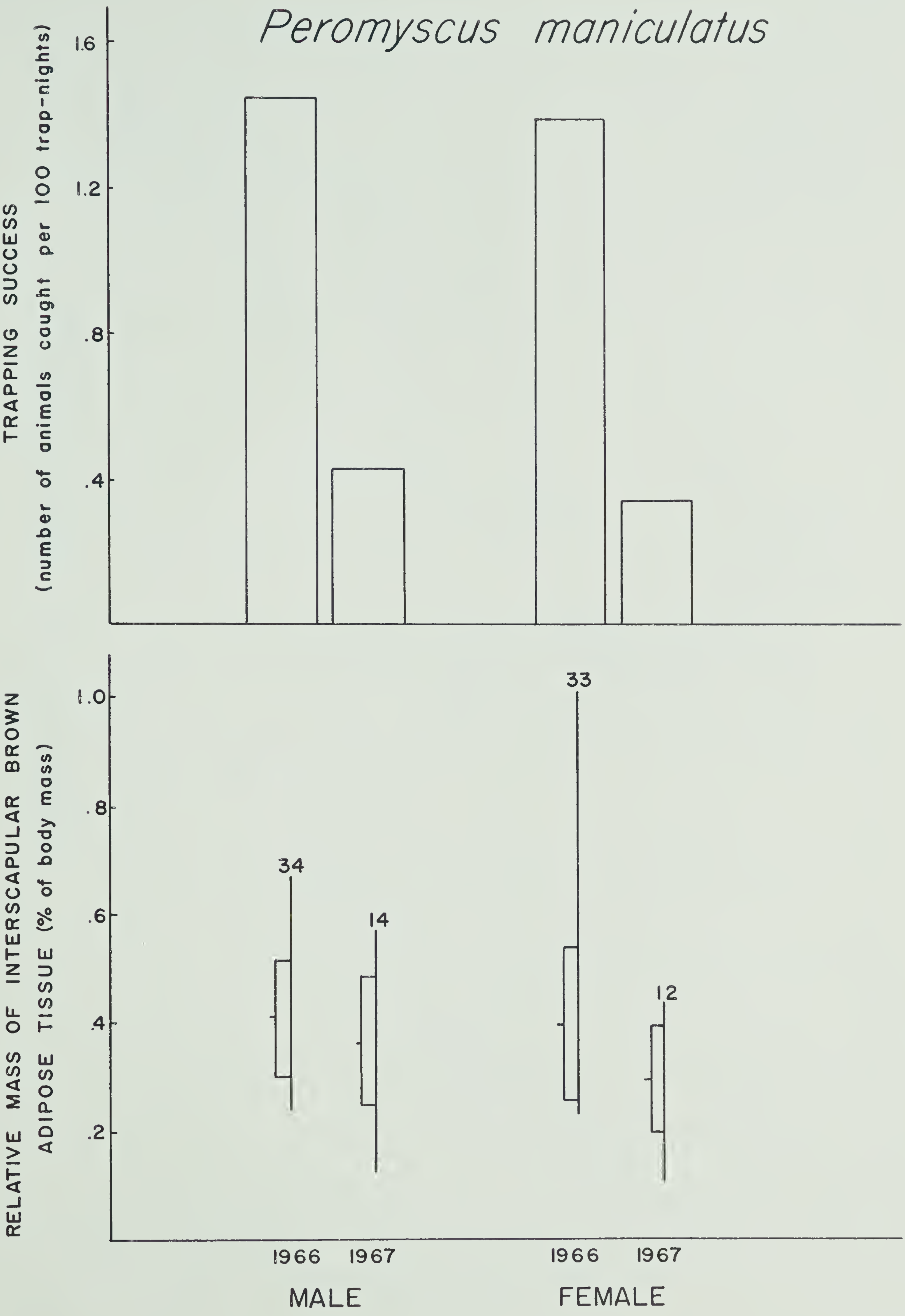
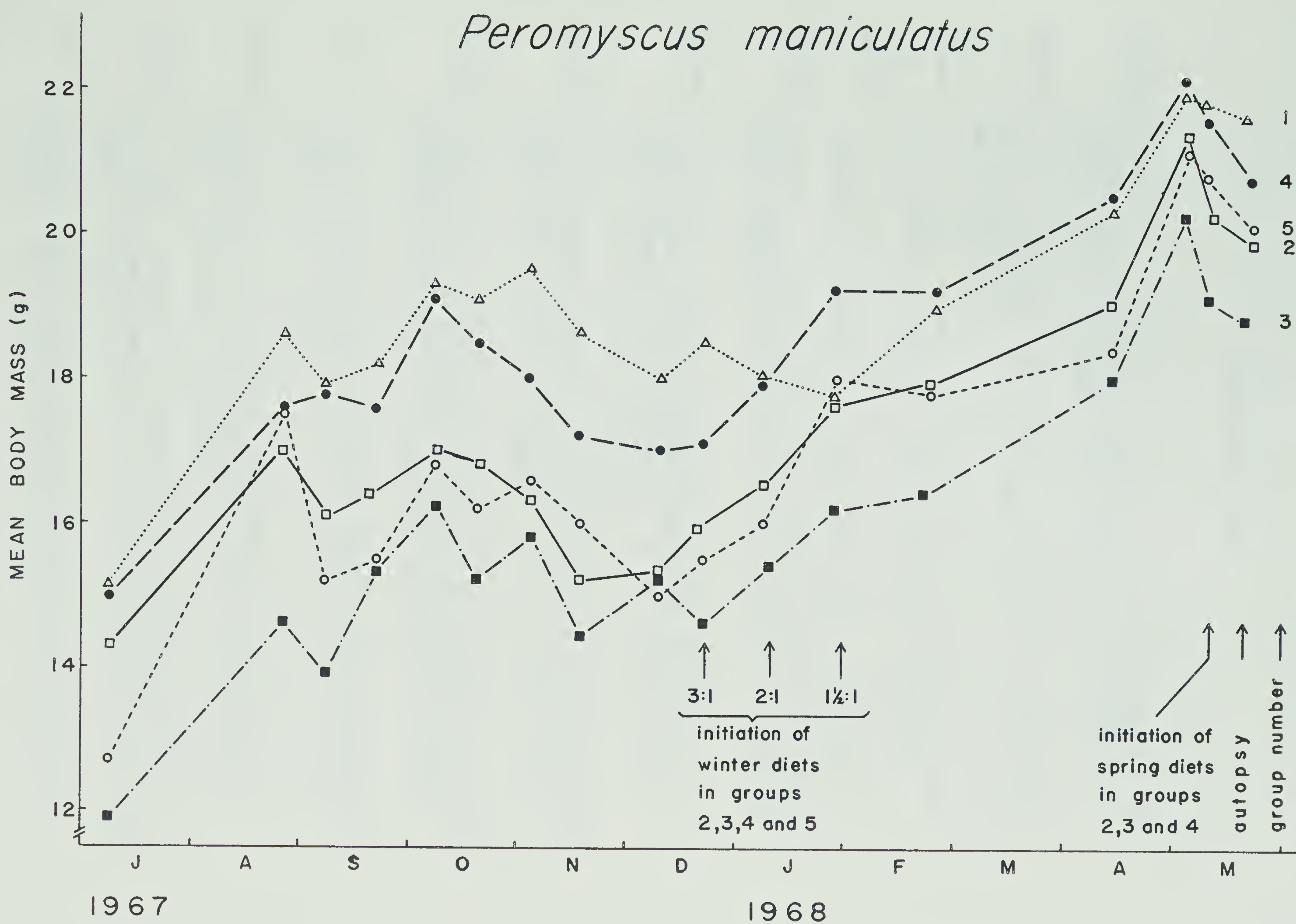

Peromyscus maniculatus
TRAPPING SUCCESS
(number of animals caught per 100 trap-nights)
1.6
1.2
.8
.4
RELATIVE MASS OF INTERSCAPULAR BROWN
ADIPOSE TISSUE (% of body mass)
1.0
.8
.6
.4
.2
34
14
33
12
1966
1967
1966
1967
MALE
FEMALE

FIGURE 16

Fluctuations in body mass for five groups of captive *Peromyscus maniculatus* submitted to diverse winter and spring diets.


Peromyscus maniculatus
MEAN BODY MASS (g)
22
20
18
16
14
12
J
A
S
O
N
D
J
F
M
A
M
1967
1968
1
4
5
2
3
3:1
2:1
1½:1
initiation of winter diets in groups 2,3,4 and 5
initiation of spring diets in groups 2,3 and 4
autopsy
group number

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